Adipose tissue deficiency, glucose intolerance, and increased atherosclerosis result from mutation in the mouse fatty liver dystrophy (fld) gene

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Abstract **The fatty liver dystrophy (*fld*) mutant mouse is characterized by neonatal fatty liver and hypertriglyceridemia that resolve at weaning, and neuropathy affecting peripheral nerve in adulthood. We now report additional significant manifestations of this single gene mutation, which include adipose tissue deficiency, glucose intolerance, and increased susceptibility to atherosclerosis. In adult *fld/fld* mice, both white and brown fat pads exhibit an 80% reduction in mass compared with wild-type controls, and consist of immature adipocytes as assessed by morphological and molecular criteria. The lack of lipid accumulation in *fld/fld* adipose tissue could be attributed, in part, to a failure to induce expression of lipoprotein lipase and enzymes involved in fatty acid synthesis, such as fatty acid synthase and acetyl-CoA carboxylase. Related to the deficiency of adipose tissue, *fld/fld* mice were also found to exhibit profound glucose intolerance, modest hyperinsulinemia, and reduced tissue response to insulin. As insulin resistance is a important risk factor in vascular disease, we examined susceptibility of *fld/fld* mice to diet-induced atherosclerosis. Mutant mice fed an atherogenic diet developed 2-fold greater aortic lesions than their wild-type counterparts, despite having a less atherogenic lipoprotein cholesterol profile. The *fld* adipose-deficient phenotype has both similarities to and distinctions from the group of rare human diseases known as lipodystrophies.—** Reue, K., P. Xu, X-P. Wang, and B. G. Slavin. **Adipose tissue deficiency, glucose intolerance, and increased atherosclerosis result from mutation in the mouse fatty liver dystrophy (*fld*) gene. *J. Lipid Res.* 2000.** 41: **1067–1076.**

Supplementary key words lipodystrophy • gene expression • insulin resistance • lipoproteins

Fatty liver dystrophy (*fld*) is a recessive mutation that arose spontaneously in the BALB/cByJ inbred mouse strain (1). Previous studies of the *fld* mutation have focused largely on two hallmark features that were apparent on its initial characterization, a fatty liver and peripheral neuropathy (2–4). Mice homozygous for the *fld* mutation (referred to throughout as *fld*) appear normal at birth, but on suckling, rapidly develop hypertriglyceridemia (1000 mg/dL) and an enlarged fatty liver characterized by accumulation of triglyceride droplets within the parenchymal cells. Accumulation of lipid in the liver is accompanied by aberrant expression patterns for several proteins including apolipoprotein A-IV, apolipoprotein C-II, hepatic lipase, and peroxisome proliferator-regulated proteins (2, 5). Remarkably, the elevated triglyceride levels in liver and blood of *fld* neonates spontaneously return to normal by the suckling-to-weaning transition at 13–18 days of age. Although this reversion coincides with the change from a lipid-rich diet to a carbohydrate-rich diet, it has been demonstrated that reversion occurs even if mice are prevented from weaning by prolonged suckling (2). This rules out the possibility that reversion of the fatty liver results from the cessation of suckling or of exposure to triglycerides or other components of mother's milk. Furthermore, the fatty liver is not reinduced by feeding adult *fld* mice a triglyceride-enriched diet (2). Together, these studies strongly suggest that formation and reversion of the *fld* fatty liver result from genetically predetermined events.

Coincident with resolution of the fatty liver between 2 and 3 weeks of age, *fld* mice develop a tremor, unsteady gait, and reduced control of the hind limbs. This neuropathy has been attributed to abnormalities in formation and maintenance of the myelin sheath in peripheral nerve (3). In contrast to the fatty liver, the neuropathy persists and progresses throughout the lifetime of the mutant animals. Another consequence of the *fld* mutation that is evident in adult animals is reduced fecundity. Males are infertile, although the reproductive organs appear normal, while females can reproduce but typically begin

Abbreviations: ACC, acetyl-CoA carboxylase; CGL, congenital generalized lipodystrophy; FAS, fatty acid synthase; *fld*, fatty liver dystrophy mutation; HDL, high density lipoprotein; HSL, hormone-sensitive lipase; LDL, low density lipoprotein; LPL, lipoprotein lipase; PPARγ, peroxisome proliferator activated receptor γ; VLDL, very low density lipoprotein.

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at a later age and generate fewer litters than wild-type mice (2, 4).

The pleiotropic phenotype exhibited by *fld* mice results from mutation at a single genetic locus, which has been mapped to mouse chromosome 12 (6). On the basis of the map position of the *fld* gene, it has been possible to exclude candidate genes encoding lipid metabolism proteins and known neurological mutants, as well as several mRNA and protein species that are expressed at abnormal levels in *fld* mouse tissues (5, 7). To obtain a better understanding of the function of the *fld* gene, we have now characterized additional manifestations of the *fld* mutation, including impaired adipose tissue development, glucose intolerance, and increased susceptibility to diet-induced atherosclerosis.

EXPERIMENTAL PROCEDURES

Animals

Nontested breeding pairs of mouse strain BALB/cByJ-*fld* were obtained from the Mouse Mutant Resource colony at The Jackson Laboratory (Bar Harbor, ME). Mice used in the studies were produced on site as offspring of +/*fld* heterozygote matings. Throughout the text, mice of the *fld/fld* genotype are referred to as "*fld*," and mice of both +/+ and +/*fld* genotypes are referred to as "wild type."

All mice were maintained in a 14-h:10-h light/dark cycle and fed Purina Mouse Chow 5001 *ad libitum*. For atherosclerosis studies, mice were fed an atherogenic diet (TD90221; Teklad Research Diets, Madison, WI) containing 75% Purina chow, 7.5% cocoa butter, 1.25% cholesterol, and 0.5% sodium cholate for 16 weeks beginning at 3–4 months of age. All animals received humane care as outlined in the *Guide for the Care and Use of Laboratory Animals*.

Adipose tissue mass and morphology

Adipose tissue mass was determined by weighing fat pads dissected from specific depots (epididymal, inguinal, and interscapular fat pads) of 10-day-old (neonatal) and 8- to 10-month-old (adult) mice. For light microscopy, epididymal and interscapular adipose tissues were excised and placed in 10% neutral buffered formalin. Tissues were then dehydrated in ethanol, embedded in paraffin, sectioned at 4 μm, stained with hematoxylin and eosin, and examined by light microscopy.

Analysis of mRNA expression levels

Mouse tissues were snap frozen at −90°C and total RNA isolated by extraction with TriZol (GIBCO-BRL, Gaithersburg, MD). Northern blots were prepared and analyzed as described (8). The cDNA probes for these studies included mouse expressed sequence tag (EST) clones from the IMAGE project for fatty acid synthase (IMAGE clone 989237; Research Genetics, Huntsville, AL) and acetyl-CoA carboxylase (IMAGE clone 850672), and cDNAs for lipoprotein lipase (9), peroxisome proliferator activated receptor γ (PPARγ) (10), and adipsin and aP2 (kindly provided by B. Spiegelman) (11).

Hormone-sensitive lipase activity

Adipose tissue homogenates were prepared and assayed for hormone-sensitive lipase activity as described (12). Briefly, epididymal fat pads from three wild-type or three *fld* mice were pooled (to obtain sufficient *fld* adipose tissue) and homogenized in buffer containing protease inhibitors, and fat-free infranatants were obtained by centrifugation at 100,000 *g*. HSL activity was determined with the diacylglycerol analog mono-oleoyl-2-*O*-mono-oleylglycerol (MOME) with incubation for 30 min at 37°C. One unit of HSL activity is equivalent to 1 μmol of fatty acid released per minute at 37°C.

Plasma lipid, glucose, and insulin measurements

Enzymatic assays for total cholesterol, high density lipoprotein (HDL)-cholesterol, triglyceride and free fatty acid levels were performed in 96-well microtiter plates with a Biomek 2000 automated laboratory workstation (Beckman Instruments, Fullerton, CA) (13). Glucose concentrations were determined colorimetrically with the Glucose Trinder reagent (Sigma Diagnostics, St. Louis, MO). Insulin was determined with a sensitive rat insulin radioimmunoassay (RIA) kit (Linco Research, St. Charles, MO).

Glucose and insulin tolerance tests

Mice were fasted 16 h and bled under anesthesia to obtain baseline glucose values. For glucose tolerance tests, mice were injected intraperitoneally with glucose dissolved in phosphate-buffered saline (2 g of glucose/kg body weight), and blood samples obtained at 15, 30, 60, and 120 min. To test for glucose-stimulated insulin secretion, mice were injected intraperitoneally with glucose and a single blood sample was taken at 60 min for insulin determinations. For insulin tolerance tests, porcine insulin (1U/kg body weight; Novo Nordisk, Basgvaerd, Denmark) was injected intraperitoneally in a volume of 100 μL, and blood samples obtained at 30 and 60 min. Glucose determinations were performed on 10 μL of plasma as described above.

Assessment of aortic lesions

After 16 weeks on the atherogenic diet, heart and aorta were removed and frozen in O.C.T. embedding compound, and lesion area in the aortic sinus measured (14).

RESULTS

Reduced body mass and impaired adipose tissue development in the *fld* mouse

In initial studies of the *fld* mutation, it was briefly noted that epididymal fat pads in suckling *fld* mice are deficient in fat (2). Indeed, *fld* mice exhibit reduced fat mass in both white and brown adipose depots and reduced body weight. At birth, *fld* mice are indistinguishable in size from wild-type littermates, but by the third postnatal day, *fld* pups exhibit significantly reduced weight gain and remain 25–30% underweight throughout their lifetime (**Fig. 1A**), despite an enlarged liver in both neonatal (2-fold enlarged compared with wild-type) and adult (20% enlarged) *fld* mice (see Fig. 1B). In contrast to the liver, inguinal and epididymal fat pad mass is reduced approximately 80% in both neonatal and adult *fld* mice. Interscapular brown adipose tissue mass is also reduced 80% in adult *fld* mice, but is not diminished in neonates. However, brown adipose tissue from both young and adult *fld* mice appears morphologically abnormal (see below). In adult mice, adipose tissue typically accounts for ~15% of body weight (15). Thus, the 25–20% reduction in *fld* body weight suggests that lean mass may also be reduced in these mice.

Morphological analysis of adipose tissue from *fld* mice at 1 and 6 months of age revealed that adipocytes are severely depleted of lipid (**Fig. 2**). Epididymal adipose sections

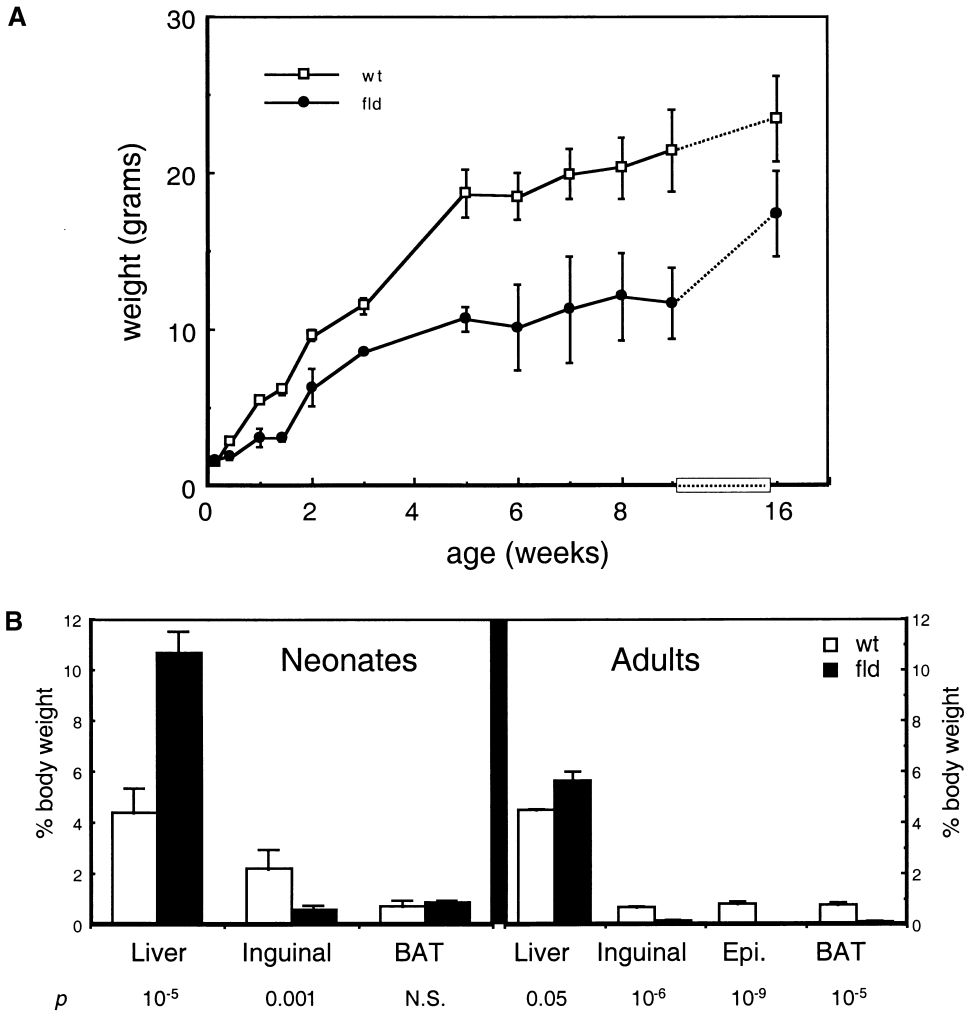

Fig. 1. Reduced body weight and adipose tissue mass in *fld* mice. (A) Body weight of wild-type and *fld* mice from 1 day to 16 weeks of age is presented as the average of 3–10 animals ± SD. Values for wild-type and *fld* were significantly different at a level of $P < 0.001$ at every age except 1 day (not significant) and at 16 weeks ($P < 0.05$). (B) Tissue weights in neonatal (10-day-old) and adult (8- to 10-month-old) wild-type and *fld* mice are expressed as percentage of body weight. Inguinal, inguinal white adipose tissue; Epi., epididymal white adipose tissue; BAT, interscapular brown adipose tissue. Inguinal and epididymal values represent mass of individual fat pads.

from 1-month-old mice exhibited immature cells containing mostly small, sparse lipid droplets (Fig. 2b). By 6 months of age, white adipocytes from *fld* mice had accumulated more lipid than at the younger age, but cells remained reduced in size and contained a heterogeneous population of lipid droplets, characteristic of incompletely differentiated adipocytes (Fig. 2c). Brown adipose tissue sections from *fld* mice also exhibited dramatically reduced lipid content at both 1 and 6 months compared with wild-type animals (Fig. 2d–f). In some regions, tissue from this depot consisted largely of muscle with small masses of lipid-poor adipocytes.

Aberrant gene expression in *fld* adipocytes

As previously described, white adipose tissue from *fld* mice expresses substantially reduced levels of lipoprotein lipase (LPL) activity (2), which may contribute to the reduced lipid accumulation in these cells. We further examined gene expression levels for several genes that are activated during normal adipose tissue development (16–19). As expected, mRNA levels for LPL were reduced in *fld* white adipose tissue, as were those for adipsin (**Fig. 3**). We also found that uncoupling protein 1 mRNA in brown adipose tissue from *fld* mice was reduced, with nearly undetectable levels in both *fld* and wild-type white adipose tissue (data not shown). In contrast, mRNA levels for PPARγ and the adipocyte fatty acid-binding protein, aP2, were markedly elevated in *fld* adipose tissue (Fig. 3). This overexpression was particularly evident in 2-week-old mice, and less prominent in older mice.

It has been demonstrated that mice carrying a knockout mutation in the LPL gene maintain normal adipose tissue mass through endogenous fatty acid synthesis (20). This suggested that the observed reduction in LPL activity

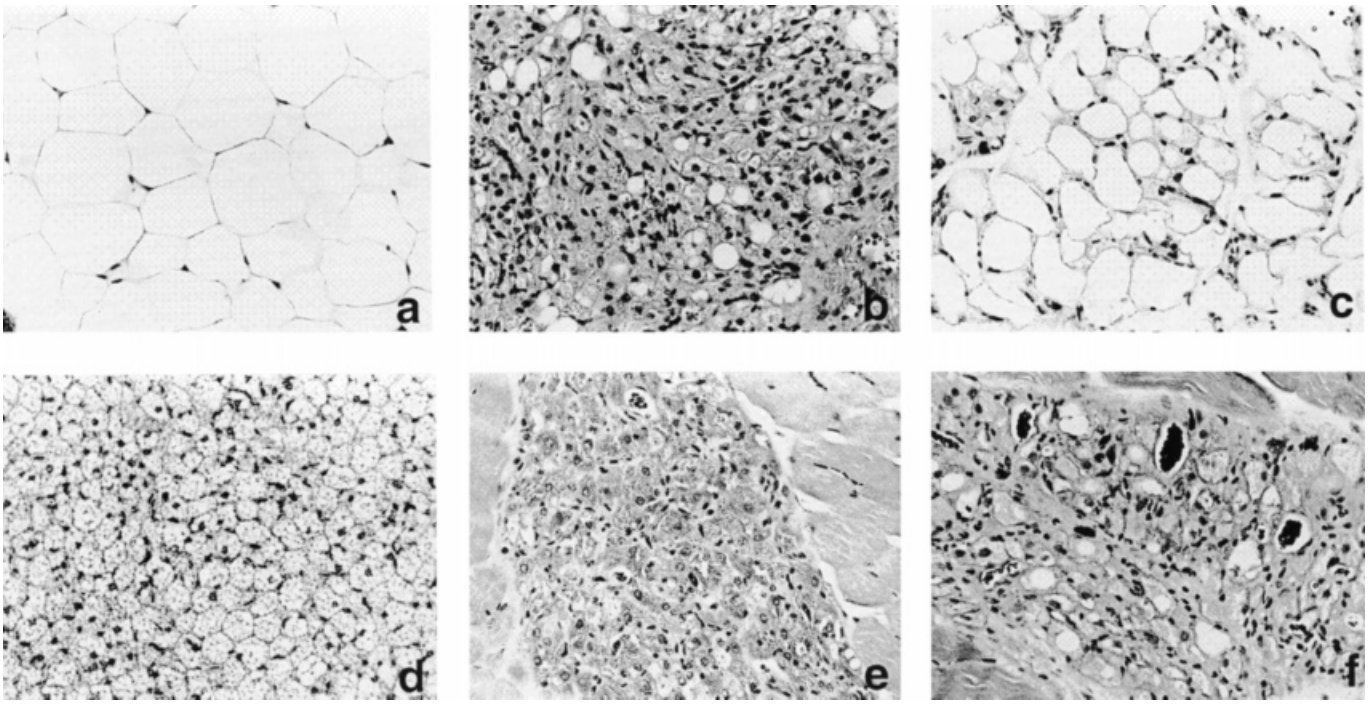

Fig. 2. Adipose tissue histology in *fld* mice. Epididymal and interscapular brown adipose tissue sections were prepared from wild-type and *fld* mice at 1 and 6 months of age, stained with hematoxylin–eosin, and viewed at an original magnification of 330×. (a) Epididymal adipose tissue from a 1-month-old wild-type mouse exhibits typical mature adipocytes with unilocular lipid droplets. (b) In contrast, cells from *fld* mice 1 month of age appear immature, with only small, sparse lipid droplets. (c) At 6 months, *fld* adipocytes contain somewhat more lipid, but are still reduced in size and often remain multilocular with numerous small lipid droplets in addition to a larger droplet. (d) Brown adipose tissue section from 1-month-old wild-type mouse showing typical lipid distribution. (e) Brown adipose tissue in 1-month-old *fld* mice contains sparse lipid droplets. (f) At 6 months of age, *fld* brown adipocytes remain severely lipid depleted.

cannot account for the reduced lipid accumulation in *fld* adipocytes. We therefore examined the mRNA expression levels for lipogenic enzyme genes in *fld* adipose tissue. Two key lipogenic enzymes, fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC), are expressed at low levels at birth and are induced ~40-fold after the rise in circulating insulin levels that occurs at weaning in normal rodents (21–24). As shown in **Fig. 4**, basal levels of FAS and ACC mRNA in both types of adipose tissue were low in wild-type and *fld* mice at 2 weeks of age (preweaning). After weaning, adipose tissue from wild-type mice exhibited a dramatic induction in both FAS and ACC expression, whereas expression levels in *fld* adipose tissues remained low. To determine whether increased lipolysis of adipocyte lipids might also contribute to the reduced lipid content, we measured hormone-sensitive lipase activity. In pooled adipose tissue extracts from 3-week-old mice, hormone-sensitive lipase activity was found to be reduced ~50% in *fld* compared with wild-type tissue (29 vs. 60 mU/mg), ruling out this possibility. These data suggest that the failure to normally activate lipogenic enzyme expression contributes to the reduced lipid accumulation in white and brown adipose tissues of *fld* mice.

Impaired glucose homeostasis in the *fld* mouse

Human subjects and animal models with severely reduced adipose tissue depots typically exhibit impaired glucose/insulin metabolism. In the case of *fld* mice, nonfasting insulin levels are elevated 2.5-fold, although glucose levels are normal (see **Table 1**). The elevated insulin levels may represent a compensatory response to maintain glucose levels, as is frequently observed in insulin-resistant humans and animals (25). To investigate this further, we performed intraperitoneal glucose and insulin tolerance tests. Results of an intraperitoneal glucose tolerance test revealed that *fld* mice exhibit severely impaired clearance of an acute glucose dose, with glucose levels remaining >400 mg/dL 2 h after glucose is administered, while wild-type mice returned to baseline levels after only 1 h (**Fig. 5A**). To assess glucose-stimulated insulin secretion, insulin levels were determined 60 min after glucose injection into a separate group of mice. Insulin levels were somewhat higher in *fld* compared with wild-type plasma [1.69 ± 0.19 ng/mL (n = 3 *fld* mice) versus 0.97 ± 0.34 ng/mL (n = 4 wild-type mice); $P < 0.05$]. Although insulin levels in *fld* mice are higher than in wild-type mice under these conditions, they are reduced from the levels seen in non-glucose-challenged mice (see Table 1). The administration of exogenous insulin together with glucose produced a typical hypoglycemic response in wild-type, but not in *fld*, mice (Fig. 5B). These results are consistent with an impairment in *fld* mice in insulin secretion, tissue insulin response, or both.

Accelerated atherosclerosis in the *fld* mouse

Epidemiological studies in humans have implicated insulin resistance and disturbances in glucose metabolism as

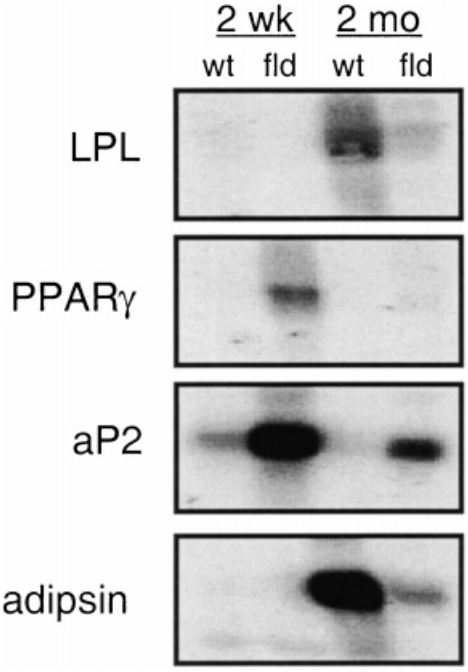

Fig. 3. Aberrant mRNA expression patterns of adipocyte markers in *fld* adipose tissue. Total RNA was isolated from the epididymal fat pads of wild-type and *fld* mice aged 2 weeks (2 wk) and 2 months (2 mo). RNA samples were pooled from 3 mice to obtain sufficient *fld* RNA and to normalize for interindividual variation. Relative levels of specific mRNA species were examined by Northern blot hybridization to cDNA probes for lipoprotein lipase (LPL), PPARγ, adipocyte fatty acid-binding protein aP2, and adipsin. Images shown were obtained by Phosphorimager analysis of blots exposed for 24 h. Longer exposures allowed detection of LPL and PPARγ mRNA in each of the wild-type samples (not shown).

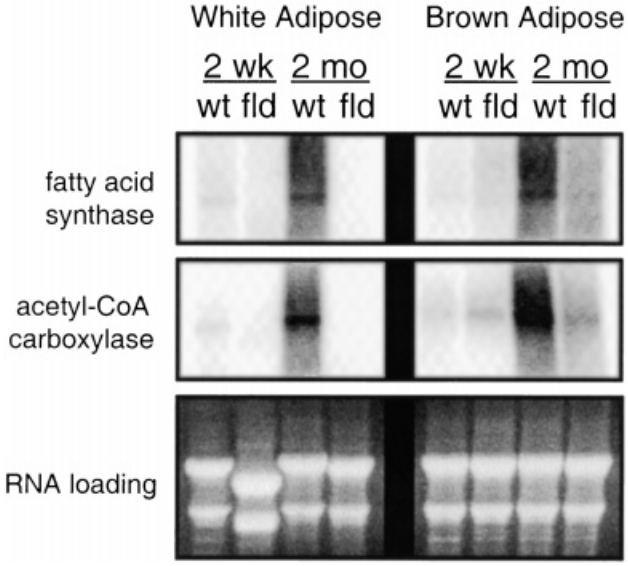

Fig. 4. Reduced expression of lipogenic enzyme mRNAs in adipose tissue from *fld* mice. Total RNA from inguinal white adipose and interscapular brown adipose tissue of wild-type and *fld* mice aged 2 weeks and 2 months was hybridized to cDNAs for lipogenic enzymes fatty acid synthase and acetyl-CoA carboxylase. The lower panel shows ethidium-stained ribosomal RNA to demonstrate equal RNA loading.

a risk factor in atherogenesis and vascular disease (26–28). We therefore investigated whether *fld* mice exhibit increased susceptibility to diet-induced atherosclerosis compared with their wild-type littermates. Baseline levels of plasma cholesterol, triglycerides, free fatty acids, glucose, and insulin were determined in female mice aged 3–4 months and consuming a chow diet. Mice were then fed an atherogenic diet for 16 weeks and lipid/glucose parameters and aortic lesions were quantitated.

The *fld* mice tolerated the atherogenic diet well, showing weight gain in parallel with the wild-type mice (data not shown). The lipid and lesion data from our studies are summarized in Table 1. On the chow diet, total cholesterol and cholesterol levels in HDL and LDL/VLDL (low density lipoprotein/very low density lipoprotein) fractions were nearly identical in *fld* and wild-type mice. Triglyceride and free fatty acid levels were also normal in *fld* mice. As noted earlier, glucose levels were normal, but insulin was elevated 2.5-fold in *fld* plasma. When fed the atherogenic diet, total cholesterol increased to similar levels for *fld* and wild-type mice. However, in *fld* mice, a greater proportion of the cholesterol was present in the HDL fraction (40 vs. 24%), and less cholesterol was present in the LDL/VLDL fraction (60 vs. 76%, *fld* and wild-type, respectively). Triglyceride and free fatty acid levels were diminished in all mice in response to the atherogenic diet, with slightly lower fatty acid levels in *fld* compared with wild-type mice. Glucose levels were similar for *fld* and wild-type mice and were not affected by feeding the atherogenic diet. In contrast, insulin levels were reduced by the atherogenic diet in both wild-type and *fld* mice, but *fld* mice maintained 4-fold higher levels than wild-type mice. Thus, the primary differences in lipid and glucose parameters between *fld* and wild-type mice fed an atherogenic diet are a less atherogenic lipid profile and elevated insulin levels in the mutant animals.

The development of aortic lesions was ascertained after 16 weeks on the atherogenic diet. The lesion areas in *fld* mice were 2-fold higher than in wild-type littermates (see Table 1). Furthermore, lesions in the *fld* mice were qualitatively more advanced than in the wild-type mice, extending beyond the aortic valve attachment points to form raised lesions in the free aortic wall (compare **Fig. 6a** and **c**). These raised lesions were detected in 92% of *fld* mice compared with only 44% of wild-type mice, indicating accelerated lesion progression in *fld* mutant mice.

DISCUSSION

The studies presented here establish that mutation in the *fld* gene leads to impaired accumulation of adipose tissue mass and insulin resistance. They further demonstrate that the *fld* mutation predisposes mice to a higher rate of diet-induced atherosclerosis compared with their wild-type littermates. These findings suggest that the *fld* mouse may serve as a naturally occurring genetic model for some

TABLE 1. Plasma lipid, glucose, and insulin levels, and aortic lesions in wild-type and *fld* mice on chow and atherogenic diets

	Chow Diet			Atherogenic Diet		
	Wild Type	*fld*	*P*	Wild Type	*fld*	*P*
Weight, g	20.4 ± 2.1	15.8 ± 1.8	<0.001	27.0 ± 1.7	23.2 ± 0.9	<0.001
Total cholesterol, mg/dL	69.5 ± 8.9	69.1 ± 4.5	NS	162 ± 43	148 ± 23	NS
HDL cholesterol, mg/dL	63.8 ± 11	58.0 ± 3.8	NS	38.7 ± 11	60.2 ± 16	<0.01
LDL/VLDL cholesterol	8.9 ± 3.2	11.6 ± 2.7	NS	124 ± 36	88.2 ± 16	<0.01
Unesterified cholesterol	26.5 ± 16	17.5 ± 1.3	NS	31.1 ± 9.7	35.9 ± 6.7	NS
Triglycerides, mg/dL	17.5 ± 28	19.9 ± 4.3	NS	2.4 ± 1.7	3.5 ± 0.9	NS
Free fatty acids, mg·dL	56.8 ± 13	46.7 ± 6.7	NS	34.1 ± 5.2	25.4 ± 5.4	<0.0005
Glucose, mg/dL	151 ± 17	156 ± 27	NS	158.0 ± 20	150.7 ± 14.1	NS
Insulin, ng/mL	1.52 ± 0.80	4.02 ± 2.32	<0.05	0.78 ± 0.40	3.33 ± 0.71	<0.005
Aortic lesions, mm^2/section	—	—	—	3,821 ± 829	7,983 ± 1,062	<0.01

Values represent the average of 8–12 mice ± SD, except aortic lesions, which are presented as ± SE. Lipid and glucose levels are given in mg/100 mL; insulin levels are given in ng/mL. Values in columns labeled *P* represent the levels of significance for differences between wild-type and *fld* mice for a specific trait as determined by Student's *t*-test; NS, not significantly different.

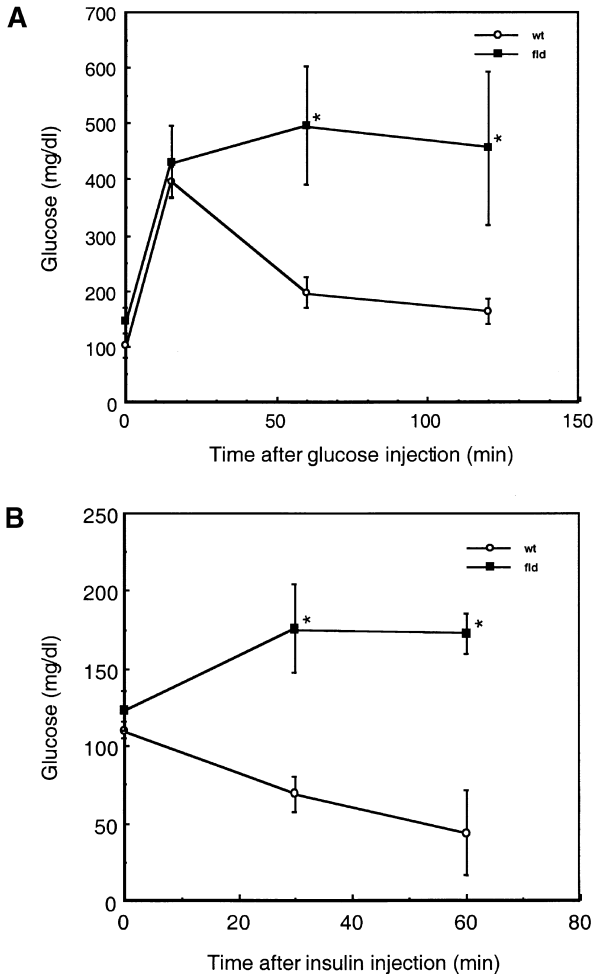

Fig. 5. Glucose and insulin tolerance tests. (A) Glucose tolerance tests were performed by administering glucose (2 g/kg) intraperitoneally to fasted mice and plasma glucose levels determined at times indicated. Each point shown represents the average ± SD for 8 adult mice (4 males and 4 females). * *fld* different from wild type at $P < 0.001$ in two-tailed Student's *t*-test. (B) Insulin tolerance tests were performed as described in (A), with the addition of insulin (1 U/kg). Each point represents the average ± SD for 4 adult female mice. * *fld* different from wild type at $P < 0.005$ in two-tailed Student's *t*-test.

aspects of human diseases such as lipodystrophy (discussed in a later section).

Reduced adipose tissue mass in *fld* mice

A combination of morphological and molecular data suggests that the reduced adipose tissue mass in *fld* mice results from a failure to form mature, fully differentiated adipocytes. The process of adipocyte differentiation has been studied extensively *in vitro* and involves the activation of a gene expression program for adipocyte transcription factors and their target genes (16–19). At the cellular level, activation of the adipocyte gene expression program is accompanied by distinctive changes in cell morphology and accumulation of cytoplasmic lipid droplets. As shown here, white adipose tissue in *fld* mice lacks large, spherical adipocytes with monolocular lipid droplets, and instead contains smaller, lipid-poor cells. At the molecular level, *fld* adipocytes exhibit aberrant gene expression profiles, with reduced mRNA levels for some adipose markers (i.e., LPL, HSL, adipsin, uncoupling protein 1, FAS, and ACC) and increased levels for others (i.e., PPARγ and aP2). Given that genes that are normally expressed both early and late in adipose differentiation exhibit altered expression levels in *fld* mice, there does not appear to be a block at a specific step in the progression of gene activation, but rather a general disordered pattern of expression. The reduced expression of both LPL and fatty acid synthetic enzymes may contribute to the lack of lipid accumulation in *fld* adipose tissue, but the mechanism by which the *fld* gene product influences their expression remains to be determined.

Given the important role of PPARγ in adipogenesis, it is noteworthy that *fld* mice exhibit a deficiency in adipocyte maturation despite high levels of PPARγ expression. It has been demonstrated that PPARγ can act as a "master regulator" to trigger adipogenesis in fibroblast cell lines in vitro, as well as to directly activate adipose-specific genes, such as aP2 and phospho*enol*pyruvate carboxykinase (29–31). In *fld* adipose tissue, the high levels of PPARγ expression are accompanied by high levels of aP2, but not by ac-

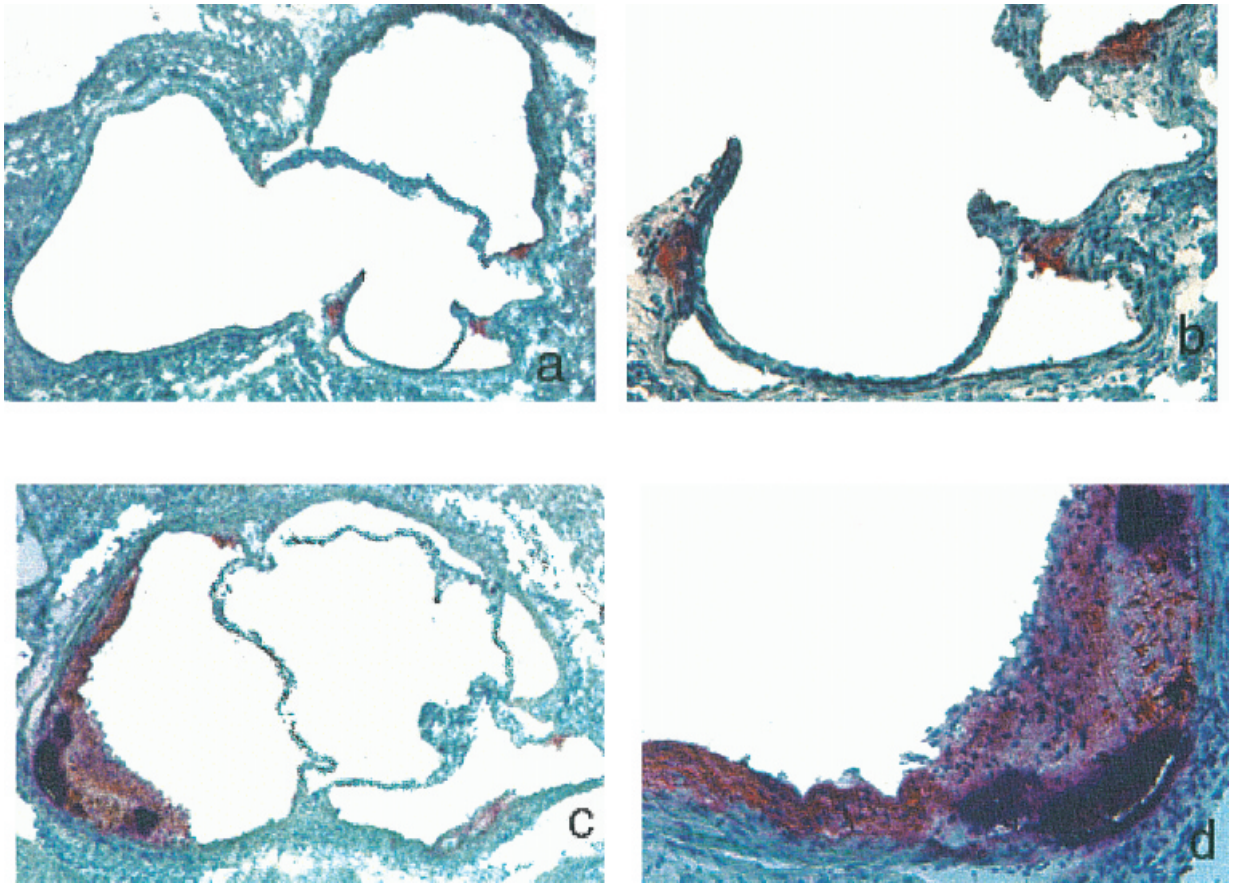

Fig. 6. Increased lipid accumulation in aortic lesions of *fld* mice. Aortic lesions were scored after 16 weeks on an atherogenic diet by staining neutral lipids with oil red O. A typical lesion from a wild-type mouse viewed at an original magnification of 40× (a) and 100× (b) shows lipid staining primarily at aortic valve attachment sites, characteristic of type I lesions. A typical lesion from an *fld* mouse viewed at an original magnification of 40× (c) and 100× (d) shows greater lesion area, extending beyond valve attachments to the free aortic wall, characteristic of type II lesions. Areas of calcification are also evident as darkly staining regions adjacent to the artery wall in (d).

celerated lipid accumulation and maturation, suggesting that the *fld* defect may lie downstream of PPARγ in the differentiation cascade. Alternatively, expression of the *fld* gene product may be required in addition to PPARγ for differentiation. An example of the latter situation is seen in mice carrying a double knockout for transcription factors C/EBPβ and C/EBPδ, which exhibit normal levels of PPARγ mRNA yet are defective in adipocyte maturation (32).

Studies presented here demonstrate that *fld* mice are glucose intolerant. Whole-body glucose metabolism is influenced by a combination of factors including glucose-stimulated insulin secretion, tissue insulin sensitivity, and hepatic glucose production (33). Our studies performed with intraperitoneal injection of glucose or insulin indicate that glucose-stimulated insulin secretion and/or tissue insulin sensitivity are impaired in *fld* mice. In support of the latter mechanism, we previously observed that insulin fails to elicit the normal response in cytoskeleton mobility in cells isolated from the inguinal fat depot in *fld* mice (7), suggesting that *fld* tissues may have reduced sensitivity to insulin. Further studies will be required to determine whether increased hepatic glucose production also contributes to the observed glucose intolerance. Given that free fatty acid levels were not elevated in *fld* mice, it appears that the mechanism underlying glucose intolerance/insulin resistance in these animals is distinct from that in type II diabetes and obesity (34). One possibility is that the lack of adequate adipose tissue mass itself contributes directly to the impaired glucose metabolism. Although human studies have established that more than 80% of insulin-mediated glucose uptake occurs in skeletal muscle (33), work in the mouse suggests that adipose tissue may also be a significant target for the hypoglycemic action of insulin. For example, mice with a muscle-specific knockout of the insulin receptor gene exhibit normal glucose tolerance, demonstrating that tissues other than muscle, most notably adipose, play a major role in glucose disposal in the mouse (35).

Atherosclerosis in the *fld* mouse

We determined that *fld* mice have increased susceptibility to diet-induced aortic lesion formation, with larger lesion areas and a greater proportion of raised lesions. Despite the increased atherosclerosis, *fld* mice exhibit lower LDL/VLDL and higher HDL cholesterol levels than wild-type mice (Table 1). While the lipoprotein cholesterol levels

would seem to indicate a less atherogenic profile, it is possible that differences in the protein constituents of lipoproteins exist in *fld* mice that could alter lipoprotein function and influence lesion susceptibility. Preliminary studies to examine levels of apolipoproteins A-I, A-II, A-IV, and E revealed slightly higher apoA-I and apoA-II levels in plasma of *fld* mice fed the atherogenic diet, in agreement with the higher HDL cholesterol levels in these animals; no differences in the levels of other apolipoproteins were observed (data not shown). Thus there are no gross differences in expected plasma apolipoprotein content, but the possibility remains that lipoproteins with altered apoprotein ratios might be present in *fld* plasma. It is also notable that *fld* mice do not exhibit hypertriglyceridemia or elevated free fatty acid levels on either a chow or atherogenic diet. This is in contrast to humans with lipodystrophies, diseases characterized by partial or complete loss of adipose tissue (see below).

The BALB/c mouse strain, including the ByJ substrain in which the *fld* mutation occurred, is relatively resistant to diet-induced atherosclerosis and produces small lesions even when fed the atherogenic diet used here containing cholate in addition to cholesterol (36, 37). However, it has been demonstrated that BALB/c mice treated with multiple low-dose streptozotocin and fed the same atherogenic diet develop aortic lesions at a substantially higher rate than non-streptozotocin-treated controls (38). The accelerated atherosclerosis in the streptozotocin-treated mice was correlated with elevated plasma glucose, resulting from destruction of insulin-producing pancreatic β cells, supporting the concept that hyperglycemia independently contributes to lesion development (38). Evidence is mounting to support a mechanism for hyperglycemia in atherosclerosis, through glycation of extracellular matrix proteins in the vascular wall and LDL particles, leading to increased atherogenicity (39–41). This is in contrast to the *fld* mouse, in which higher lesion scores are not associated with hyperglycemia, and with only modest hyperinsulinemia. The mechanism for increased atherosclerosis in *fld* mice in the absence of known risk factors such as hyperlipidemia and hyperglycemia remains to be determined. It would also be interesting to determine whether *fld* mice also develop lesions on a non-cholate-containing diet.

Relationship of the *fld* defect to human lipodystrophies

The phenotype of the *fld* mutant mouse has similarities to the group of rare disorders in humans known as lipodystrophies. Human lipodystrophies are a genetically heterogeneous group of diseases characterized by a generalized or partial loss of body fat that is apparent from birth or develops in adulthood (42–46). The *fld* mouse shares some features, but not others, present in congenital generalized lipodystrophy (CGL). Most notably, CGL patients exhibit paucity of fat in subcutaneous and other adipose tissue depots from birth, and as we have shown here for *fld* mice, fat cells are present in CGL patients but contain little fat (43). In CGL, the adipose deficiency typically is accompanied by severe insulin resistance, hyperinsulinemia, hyperglycemia, hypertriglyceridemia, and fatty liver (43). The *fld* mice develop modest hyperinsulinemia, but unlike CGL patients do not develop hyperglycemia. In addition, the fatty liver and hypertriglyceridemia in *fld* mice are restricted to the neonatal period, whereas CGL patients may have fatty liver and high, but variable, triglyceride levels throughout their lifetime. Interestingly, of 10 CGL patients studied for several years, free fatty acid levels were in the normal range as was seen in the *fld* mouse (43); however, other studies have reported elevated free fatty acid levels in lipodystrophy patients (47, 48). Other similarities between lipodystrophy syndromes and the *fld* mutant mouse include reports of reduced fertility in CGL (43); however, fertility is not impaired in a related disorder known as lipoatrophic diabetes, despite the lack of adipose tissue and low leptin levels (49). Peripheral neuropathy, an invariant and progressive manifestation of the *fld* mutation, has been reported in numerous lipoatrophic diabetes patients (50–52).

Transgenic and knockout models with reduced adipose tissue have been reported. These include mice expressing a toxin transgene that ablates adipose tissue, mice carrying null alleles for the C/EBP transcription factors, mice expressing a dominant-negative transcription factor that interferes with function of endogenous factors required for adipocyte development, and mice expressing an adipocyte-specific transgene for the SREBP1c transcription factor involved in regulation of fatty acid synthesis (53–56). In addition to reduced or absent adipose tissue, these models resemble lipodystrophies in the presence of hypertriglyceridemia, fatty liver, and insulin resistance, often progressing to diabetes. Shimomura and colleagues (57) have demonstrated in the SREBP1c transgenic mouse that low-dose leptin infusion reverses the insulin resistance and diabetes, but does not restore adipose tissue. Given that leptin mRNA levels are reduced to ~10% of normal in *fld* mice (K. Reue, unpublished observation), it would be interesting to see whether glucose intolerance and atherosclerosis susceptibility improve in response to leptin infusion.

The *fld* gene resides on mouse chromosome 12 (6). This map position indicates that it is distinct from the single known human lipodystrophy gene, that for lamin A/C, which is mutated in familial partial lipodystrophy (58, 59). Interestingly, a mouse atherosclerosis susceptibility locus, *Ath6*, has been mapped to proximal chromosome 12 (60); however, a comparison of genetic markers flanking the two genes makes it possible to exclude *fld* as a candidate for *Ath6* (B. Paigen, personal communication, 1999). On the basis of the map position of the *fld* gene, it is also possible to exclude known genes encoding proteins involved in adipocyte development and lipid metabolism. Using a positional cloning strategy, we have isolated a candidate for the *fld* gene that appears to be novel and unrelated to previously identified proteins (61). The availability of this gene should facilitate the elucidation of the *fld* protein function, including its role in adipogenesis, glucose metabolism, and atherosclerosis.

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